SUGARS AND SUGAR PRODUCTS

Sensitive Thin Layer Chromatographic Detection of High Fructose Corn Sirup and Other Adulterants in Honey

IRENE KUSHNIR
U.S. Department of Agriculture, Science and Education Administration, Eastern Regional Research Center, Philadelphia, PA 19118

A highly sensitive procedure has been developed to detect the undeclared addition of high fructose corn sirup (HFCS) to honey. Carbohydrates must be separated first to achieve the requisite degree of sensitivity: charcoal-Celite chromatography was used to isolate a fraction containing oligo- and polysaccharides. The fraction was then concentrated and examined by thin layer chromatography on silica gel. Pure honeys yielded only 1 or 2 blue-grey or blue-brown spots at R_f values >0.35; a series of spots or blue streaks extending from the origin characterized adulterated samples. The method detects HFCS and conventional honey adulterants at levels as low as 10% or less of the total mixture. In addition, the procedure detects the presence in honey of all starch-derived sugar sirups tested thus far, regardless of the plant source.

As a natural product of limited supply and frequently high price, honey has traditionally been a target for adulteration. Existing methods for detecting adulteration were adequate for maintaining the integrity of the honey market until the advent of high fructose sirups. Advances in bound enzyme technology have enabled the starch-processing industry to convert glucose to sweeteners containing glucose and fructose. The ratio of these 2 sugars in these sweeteners is such that when the sweeteners are mixed with honey, the glucose-fructose ratio remains within the compositional range for honey. In the United States, corn has been used almost exclusively as the starch source for the production of such sirups. High fructose corn sirup (HFCS) is typically composed of 50% glucose, 42% fructose, and 8% higher saccharides, including trace amounts of polysaccharides (1). The low cost of this sweetener, its highly refined nature, and its resemblance to honey in major sugar components make it a potential adulterant whose presence in honey is very difficult to detect. Moreover, the natural variability of honey composition increases the complexity of the problem of detection.

An unequivocal method recently adopted as official first action for HFCS in honey is based on stable carbon isotope ratio analysis (2). Because this procedure is expensive, requires highly sophisticated instrumentation, and can be conducted by only a very few laboratories, a method was needed which could be used in field laboratories by regulatory agencies. The thin layer chromatographic (TLC) procedure described here and the gas-liquid chromatographic (GLC) method (3) were developed simultaneously in our laboratory to meet this need.

Preliminary work consisted of subjecting HFCS samples to hollow fiber filtration to isolate and characterize polysaccharides and dextrins present in the sirup. Molecular weights were then determined by gel filtration. The results provided evidence for the presence in HFCS of both high molecular weight carbohydrates and malto-dextrins of varying molecular weight. This provided the basis for the development of a TLC method for detecting HFCS adulteration of honey.

The method presented in this paper falls within the expertise and capabilities of an ordinary quality control or regulatory agency laboratory.

METHOD

Description of Samples

Domestic honey producers supplied nearly 500 honey samples, accompanied by certificates of authenticity; 290 of these samples (representing 41 floral sources) and 11 samples of honeydew honey (identified by positive polarization) were analyzed, as well as HFCS samples from 7 United States manufacturers. Forty-four samples of suspicious commercial honey were also tested for adulteration.

Sample Preparation

Charcoal column.—Use mixture of equal parts of Darco G-60 charcoal and a rapid diatomaceous filter aid (Celite 545 or Dicalite 4200). A column ca 20–22 mm × 370 mm with 1 L spherical section and 35/20 spherical ground joint is satisfactory (4). Pack plug of fine glass wool in base of column; close outlet tube and partly fill column with water. Open outlet and let water flow through column to remove any air bubbles. Close outlet when water level is ca 10 mL above glass wool and then add slurry of filter aid sufficient for ca 1 cm depth and let settle under gravity. Pour in slurry of 12 g charcoal/filter aid in 150 mL water. Drain 5 min, apply 4 psi pressure until surface stabilizes, and then increase to 10 psi. Clean excess charcoal from glass surfaces by suction, and add slurry of filter aid sufficient to give 1–2 cm layer. Wash column with 500 mL water and 200 mL 50% ethanol, under which it may be stored. Before use, wash column with 250 mL water. Vacuum operation may be used, although pressure is preferred. A typical flow rate is 8.5 mL/min at 10 psi.

Weigh (to 1 mg) 1 g sample in 30 or 50 mL beaker, add 10 mL water, and place on top of column. Force into column by suction, but do not let column run dry. Use two 5 mL portions of water to transfer residues to column. Wash with 300 mL 7% ethanol, which is discarded, and subsequently with 100 mL 50% ethanol. Evaporate this eluate in tared 50 mL beaker on steam bath in a current of air or nitrogen and dry in vacuum oven for ½ hr at 65°C, and weigh residue.

Transfer residue to small test tube (13 × 100 mm is adequate) with a total of 1 mL water, and evaporate to dryness in 60°C bath in current of air or nitrogen. Dissolve residue in 0.1 mL water for each 10 mg material.

Thin Layer Chromatography

Plates.—20 × 20 cm (Analtech) coated with 250 μm thick silica gel G.

Solvent.—*n*-Butanol-acetic acid-water (2+1+1).

Reagent.—Dissolve 1 mL redistilled aniline and 1 g diphenylamine.HCl in 50 mL acetone and add 5 mL 85% H_3PO_4. Prepare fresh daily or store below 0°C.

Procedure.—Place solvent in tank 15 min before inserting plate. Apply 2 and 6 μL (in three 2 μL portions) of test solution to plate. Apply samples of authentic and adulterated honey (prepared as above) to each plate as controls. These solutions may be preserved by freezing or drying. Place in developing tank until solvent front approaches upper edge. Remove, dry, and spray thoroughly with reagent.

After acetone has evaporated, place plate in 90–95°C oven until spots are well developed, ca 10–15 min.

Store TLC plates in desiccator. TLC procedure is sensitive to ambient relative humidity; therefore, when humidity is high, use 5 × 20 cm plates to minimize time of exposure to the humidity during spotting of plates.

Interpretation

Pure honey will show 1 or 2 large blue-gray or blue-brown spots at R_f >ca 0.35. When weight of isolated carbohydrate fraction is ≤15 mg (1.5%), any blue streaks or series of spots extending from origin provide conclusive evidence of adulterants. If weight is >15 mg, a second test must be conducted to confirm adulteration (see Results and Discussion).

Results and Discussion

Alcohol precipitation is the most commonly used method to isolate dextrins and polysaccharides from sugar-containing products. Carbon column chromatography, however, was chosen because it was necessary to minimize interference caused by dextrins and other materials present in honeydew honey. Moreover, depending on its commercial source and the refining process used in its production, high fructose corn sirup (HFCS) may contain only trace amounts of polysaccharides. The carbon column procedure provides the sensitivity required to assure the applicability of the test even when adulterants are present as less than 10% of the mixture.

Seven samples of HFCS from different United States manufacturers were analyzed according to the described procedure. The average content for higher sugars was 1.22% and the range, 0.8–2.8%. Because polysaccharides are present in HFCS in only trace amounts, the range is due primarily to the dextrin content (see Fig. 1).

To ascertain whether dextrins and polysaccharides in HFCS are affected by enzymes in honey, a time study was conducted. HFCS samples were reduced to a moisture level comparable to that of honey and mixed with honey with a diastase number of 29, which is above the

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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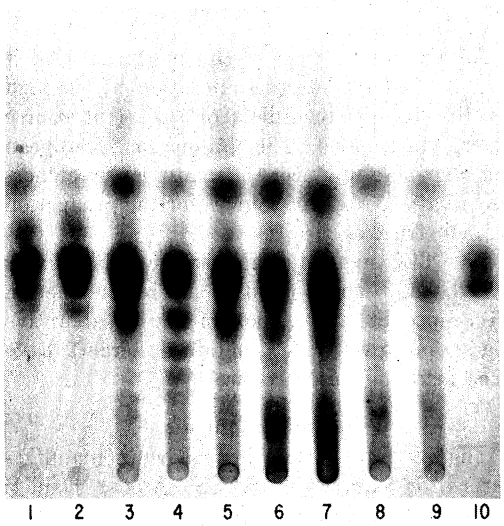

FIG. 1—Photograph of TLC plate showing resolution of oligosaccharides and higher molecular carbohydrates of concentrates from honey and HFCS isolated by charcoal column chromatography eluted with 50% ethanol.

1, 2 μL concentrate from orange honey; 2, 2 μL concentrate from clover honey; 3, 6 μL concentrate from admixture of 5% HFCS and honey; 4–6, 2 μL concentrates from admixtures of 10, 25, and 50% HFCS and honey, respectively; 7, 2 μL concentrate from admixture of 5% conventional corn sirup and honey; 8–9, 2 μL concentrate from HFCS from 2 manufacturers; 10, 2 μL mixture containing 5 μg raffinose and 5 μg melezitose.

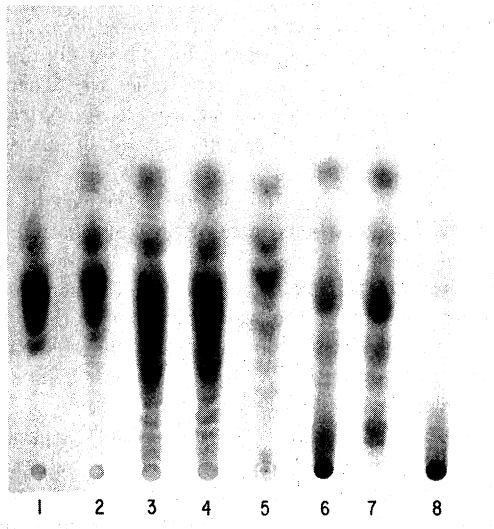

FIG. 2—Photograph of TLC plate showing resolution of oligosaccharides and higher molecular carbohydrates of concentrates from honey and HFCS isolated by charcoal column chromatography eluted with: a, 50% ethanol; b, 25% ethanol; and c, 50% ethanol after elution with 25% ethanol.

1, 2 μL concentrate eluted with a from alfalfa honey; 2, 2 μL concentrate eluted with a from buckwheat honey; 3, 2 μL concentrate eluted with a from honeydew honey; 4, 2 μL concentrate eluted with b from honeydew honey; 5, 2 μL concentrate eluted with c from honeydew honey; 6, 2 μL concentrate eluted with a from HFCS; 7, 2 μL concentrate eluted with b from HFCS; 8, 2 μL concentrate eluted with c from HFCS.

average diastase value (20.8) of honey (4). After 6 months' storage at room temperature, no degradation of HFCS polysaccharides had occurred, as indicated by the procedure described here.

After being subjected to carbon column chromatography, 290 samples of pure honey were analyzed by the thin layer chromatographic (TLC) procedure. As evident from Fig. 1, pure nectar honeys do not contain carbohydrates which produce spots at R_f values of 0.35 or less. Some honeys which contain honeydew, however, do contain interfering sugars which produce spots or streaks within this region. Of the 290 samples analyzed, 15.2%—that is, 44 samples—showed interfering sugars, at an average level of 2.79% with a range of 1.65–6.35% (see Fig. 2).

As indicated in Fig. 3, 60% of all United States honeys analyzed in a comprehensive survey (4) contain 1.5% or less higher sugars. In this 1962 analytical survey of 490 samples of honey from 47 States, representing 82 single

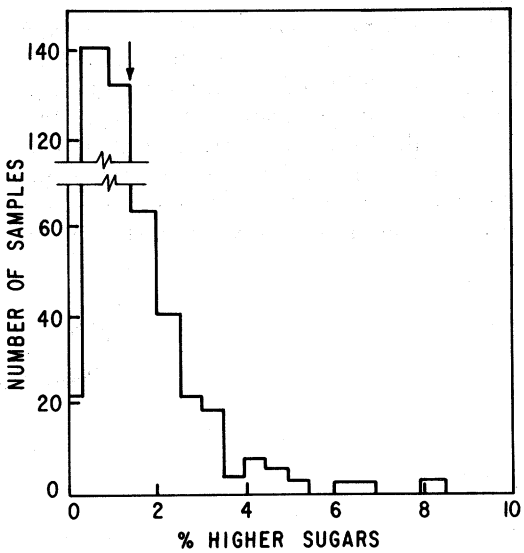

FIG. 3—Distribution of higher sugars among 490 United States honey samples analyzed in a comprehensive survey in 1962 (4).

floral types and 93 blends of known composition, the higher sugar contents ranged from 0.13 to 8.49. Comparable values have been obtained for foreign honeys in similar studies (5). Therefore, the weight of the isolated carbohydrate fraction can be used as an indicator of suspect samples.

As has already been noted, if the weight of the isolated carbohydrates from the column is equal to or less than 15 mg, the presence of adulterants is conclusive, and no further tests are necessary. If the weight is greater than 15 mg, the charcoal column pretreatment should be repeated in the following manner: Prepare the sample as described, with the exception of washing the column with 100 mL 25% ethanol, which is discarded, before proceeding with the 50% ethanol. Evaporate this eluate in a 50 mL beaker as described. Transfer this residue quantitatively to a small test tube and evaporate to dryness. Dissolve the residue in 0.1 mL water. Because only trace amounts of polysaccharides are present, the sides of the tube should be washed carefully with 0.1 mL water to dissolve all precipitate. All oligosaccharides contained in honeydew honeys will be eluted with 25% ethanol. Thus, any blue streaks or series of spots extending from the origin on the TLC plate following this treatment will provide conclusive evidence of adulteration, as demonstrated in Fig. 2.

Four adulterated honey samples containing 5, 10, 25, and 50% HFCS were prepared and one was prepared with 5% conventional corn sirup. As indicated in Fig. 1, adulterated samples produce streaks (blue) or a series of spots extending from the origin. The TLC procedure was also applied to a total of 44 samples of suspicious commercial honey. Adulterations by HFCS, conventional corn sirup and invert sugar were identified.

An added advantage of this method is that it can be used to detect adulteration by all high fructose sirups, regardless of the plant source of the starch used in its production. European and Japanese producers of industrial sweeteners use a variety of plant sources for the production of high fructose sirups.

The TLC method, which was collaboratively tested, has been adopted as official first action (6). Since the completion of the collaborative study, improvements (contained herein) have been made in the procedure.

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